Journal of Medical Virology 58:408–412 (1999)

Genome Type Analysis of Brazilian Adenovirus Strains of Serotypes 1, 2, 3, 5, and 7 Collected Between 1976 and 1995

Adriana E. Kajon,[1] Silvana A.R. Portes,[2] Wyller A. de Mello,[3] Jussara P. Nascimento,[2] and Marilda M. Siqueira[2]*

[1]Departamento de Microbiologia, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Departamento de Virologia, FIOCRUZ, Instituto Osvaldo Cruz, Rio de Janeiro, Brazil
[3]Departamento de Virologia, Instituto Evandro Chagas, Belem, Para, Brazil

A collection of 92 epidemiologically unrelated isolates of Ad1 (n = 14), Ad2 (n = 29), Ad3 (n = 19), Ad5 (n = 16), and Ad7 (n = 14) collected in the cities of Belem do Pará (1°S 48°W) and Rio de Janeiro (23°S 43°W) between 1976 and 1995 from patients with respiratory disease and conjunctivitis were characterized by restriction enzyme analysis of genomic DNA. Among the strains of subgenus B, two different genome types of serotype 7, 7b and 7e, were identified. The analysis of their temporal distribution throughout the study period suggested an alternating appearance of these two DNA variants. Only one genome type of Ad3, 3p, was detected during the sampling period. Further analysis with Xba I, Bcl I, and Hpa I indicated that it is a p1-like genome type. Both previously described and new genomic variants were identified among subgenus C strains. Genome types D1, D7, D10, and one not previously described were identified among the 14 Ad1 strains analyzed. Genome types D2, D5, D25, and 13 new DNA variants were identified among the 29 Ad2 isolates. Genome type D38 and 5 new variants were found among the 16 strains of Ad5. In spite of the relatively small size of the sample analyzed, the results of this study confirm the important genetic variability previously observed for members of subgenus C by other authors. ***J. Med Virol. 58:408–412, 1999.***

KEY WORDS: restriction enzyme analysis; molecular epidemiology; respiratory infections

INTRODUCTION

Adenoviruses have been recognized as important human pathogens since their discovery by Rowe et al. in 1953. So far, 49 serotypes have been identified and classified within 6 subgenera, A to F, based on relative nucleic acid homology, fiber protein characteristics and other biochemical and biological properties [Schnurr and Dondero, 1993; Shenk, 1996]. Adenovirus 3 and 7 (Ad3 and Ad7), classified within subgenus B, are a frequent cause of respiratory and ocular infections in man. Subgenus C adenoviruses—especially types 1, 2, and 5—represent some of the most frequently isolated human adenovirus serotypes all over the world. These serotypes are common etiologic agents of respiratory disease, mainly in children under 5 years of age, and account for more than half of all isolates reported by the World Health Organization between 1967 and 1976 [Schmitz et al., 1983].

The genomic characteristics of subgenus B and C strains, circulating in different geographic areas, have been studied by several groups, but still relatively little is known about the genetic variability and molecular epidemiology of adenovirus infections in the north of South America and the Caribbean. Several reports have shown the important role of adenovirus infections in severe and life-threatening respiratory disease of children in South America [Kajon and Vicente, 1990; Wu et al., 1990; Garcia et al., 1993; Murtagh et al., 1993; Murtagh and Kajon, 1997]. A study conducted in Rio de Janeiro showed adenoviruses to be the second major virus to infect the respiratory tract of the local children population [Nascimento et al., 1991].

In this article we present the results of the genomic characterization by restriction enzyme analysis of 33 epidemiologically unrelated isolates of subgenus B and 59 isolates belonging to subgenus C collected between

Dr. Kajon is currently at the Department of Genetics, University of Georgia, Life Sciences Building, Athens, GA 30602-7223.

*Correspondence to: Dr. Marilda M. Siqueira, Departamento de Virologia, Instituto Oswaldo Cruz, Av Brasil 4365, Manguinhos, Rio de Janeiro RJ 21045-900, Brazil. E-mail: mmsiq@ioc.fiocruz.br

Accepted 12 January 1999

1976 and 1995 in the Brazilian cities of Rio de Janeiro and Belem do Pará.

MATERIALS AND METHODS

Virus Strains

Adenovirus strains were isolated from nasopharyngeal aspirates, throat swabs, or conjunctival swabs of patients with acute respiratory infection or conjunctivitis in the Brazilian cities of Belem do Pará (1°S 48°W) and Rio de Janeiro (23°S 43°W), as listed in Tables I and II. Adenovirus isolates of serotypes 3 and 7 were obtained from both children and adults, but isolates of subgenus C were obtained only from children under 5 years of age. All isolated strains were serotyped by neutralization with rabbit reference antisera. Viral isolation and serotyping of the strains from Belem was carried out at the Department of Virology of the Evandro Chagas Institute. The clinical samples collected in Rio de Janeiro were processed at the Department of Virology of the Oswaldo Cruz Institute.

DNA Restriction Analysis and Genome Type Denomination

Intracellular viral DNA was prepared from infected Hep-2 cells grown in 25 cm^2 plastic flasks by the method described by Shinagawa et al. [1983]. Aliquots containing 1–2 μg of DNA purified from subgenus B strains were initially digested with BamHI and SmaI with 10 units of enyzme according to manufacturer specifications (Boehringer, Mannheim, Germany). Strains of serotype 3 were further analyzed by cleavage with XbaI, Bcl I, and Hpa I. Digested DNA was analyzed in horizontal 1.2% agarose gels. Aliquots of purified DNA (1–2 μg from subgenus C strains were digested with 10 units of endonucleases BamHI, Bgl II, BstEII, EcoRI, HindIII, KpnI, and SmaI according to manufacturer specifications.

Digested DNA was analyzed in horizontal 1.2% agarose gels. Restriction profiles were examined in a transilluminator and photographed using Polaroid Land film 667 or 57. Genome types of subgenus B were denominated according to the system proposed by Li and Wadell [1986], whereas genome types of subgenus C were denominated according to the system proposed by Adrian et al. [1985]. Enzyme codes for restriction profiles were obtained from the literature when available [Adrian et al., 1990].

RESULTS

Subgenus B

A collection of 19 epidemiologically unrelated adenovirus strains of serotype 3 and 14 strains of serotype 7 isolated between 1976 and 1994 in the cities of Belém do Pará and Rio de Janeiro from patients with conjunctivitis and respiratory disease were studied by restriction enzyme analysis of genomic DNA with *Bam*HI and *Sma*I. Although two different genome types of Ad7, 7b and 7e, were identified among the studied strains, only one DNA variant of Ad3, 3p, was detected throughout the 18-year period. Further analysis with Xba I, Bcl I, and Hpa I indicated that it is a p1-like genome type [Li and Wadell, 1988]. Restriction profiles of genome types belonging to subgenus B are shown in Figure 1 and results summarized in Table I.

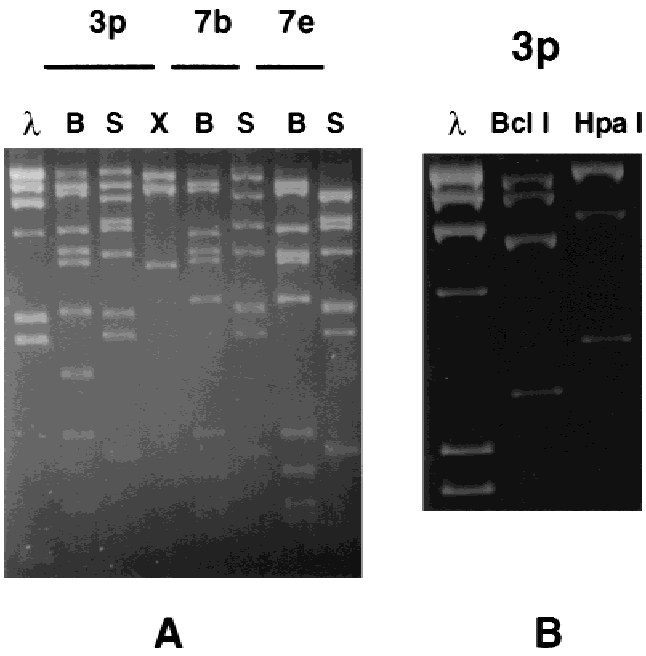

Fig. 1. Restriction profiles of adenovirus strains of subgenus B. **(A)** B: Bam HI, S: Sma I X: Xba I. **(B)** Bcl I and Hpa I restriction patterns of Ad3p. Lambda DNA digested with Hind III was used as a molecular weight marker.

Subgenus C

A collection of 59 epidemiologically unrelated strains of hAd1 ($n = 14$), hAd2 ($n = 29$), and hAd5 ($n = 16$) of subgenus C isolated in the cities of Belem do Pará and Rio de Janeiro from the nasopharyngeal aspirates or throat swabs of patients with respiratory disease were characterized by restriction enzyme analysis of genomic DNA with endonucleases *Bam*HI, *Bgl* II, *Bst*EII, *Eco*RI, *Hind* III, *KpnI,* and *Sma*I and typed according to the system proposed by Adrian et al. [1985]. Both previously described and new genomic variants were identified. The enzyme codes, year, and place of isolation of all genome types are presented in Table II. Novel restriction profiles **(New I–VI)** obtained with BamHI, BstEII, HindIII, and KpnI are shown in Figure 2.

Genome types D1, D7, D10, and one not previously described DNA variant designated **new a** were identified among the 14 Ad1 strains analyzed. Genome types D2, D5, D25, and 13 new DNA variants designated **new a–m** were identified among the 29 Ad2 isolates. Genome type D38 and 5 new variants (**5*, 5a–d**) were found among the 16 strains of Ad5. New genomic variants resulted from novel combinations of previously described restriction profiles (2a, 2e–g, 2j–m, 5*, 5a–c) as well as from cleavage patterns not previously described (1a, 2b–d, 2h–i, and 5d).

TABLE I. Origin of Ad3 and Ad7 Isolates and Associated Clinical Signs

Serotype	Isolate no.	Origin	Year	Patient			Genome type
				Age	Sex	Clinical signs	
3 (n = 19)	14865	Belem	1976	39 y	F	Conjunctivitis	3p1
	18253	Belem	1979	30 y	M	Conjunctivitis	3p1
	22798	Belem	1982	6 m	F	ARI	3p1
	25800	Belem	1983	28 y	M	Conjunctivitis	3p1
	78/83	Rio de Janeiro	1983	1 y	F	ARI	3p1
	217/83	Rio de Janeiro	1983	25 y	F	Conjunctivitis	3p1
	448/83	Rio de Janeiro	1983	3 m	F	ARI	3p1
	27422	Belem	1984	33 y	M	Conjunctivitis	3p1
	504/84	Rio de Janeiro	1984	NA	M	ARI	3p1
	28243	Belem	1985	34 y	F	Conjunctivitis	3p1
	30194	Belem	1986	5 y	F	Conjunctivitis	3p1
	30753	Belem	1986	3 m	F	ARI	3p1
	30774	Belem	1986	9 y	F	ARI	3p1
	30786	Belem	1986	3 y	F	ARI	3p1
	30787	Belem	1986	5 y	F	ARI	3p1
	30805	Belem	1986	2 a	F	Conjunctivitis	3p1
	31096	Belem	1987	9 m	F	Conjunctivitis	3p1
	239/88	Rio de Janeiro	1988	NA	M	ARI	3p1
	53301	Belem	1994	2 y	M	ARI	3p1
7 (n = 14)	15235	Belem	1976	3 m	M	ARI	7e
	24088	Belem	1983	4 y	M	ARI	7e
	24106	Belem	1983	49 y	M	Conjunctivitis	7e
	24553	Belem	1983	1 y	F	ARI	7b
	225/83	Rio de Janeiro	1983	6 m	F	ARI	7b
	24860	Belem	1983	NA	M	ARI	7e
	25462	Belem	1983	4 y	F	ARI	7b
	26970	Belem	1984	5 y	F	Conjunctivitis	7b
	28568	Belem	1985	NA	F	Conjunctivitis	7b
	30711	Belem	1987	41 y	M	Conjunctivitis	7b
	32584	Belem	1987	8 y	F	ARI	7e
	33139	Belem	1987	4 y	F	ARI	7e
	213/88	Rio de Janeiro	1988	2 y	F	ARI	7b
	51916	Belem	1994	7 m	F	ARI	7b

ARI, acute respiratory infection; NA, not available.

DISCUSSION

Before the present study, only a few reports in the literature had described the characterization by restriction enzyme analysis of adenovirus strains of serotypes 1, 2, 3, 5, and 7 isolated in Brazil [Gomes et al., 1989; Niel et al., 1991; Harsi et al., 1995]. The source of most of those isolates was stools so this represents the first large collection of Brazilian adenovirus isolates obtained from the respiratory tract characterized so far.

The data presented here were obtained from a collection of already isolated strains available at the Oswaldo Cruz and the Evandro Chagas institutes and not from all cases of Ad infection analyzed by these institutions during the study period. Therefore they do not reflect the impact of adenovirus infections in the communities of Belem and Rio de Janeiro but the local genetic variability of Ad strains.

With the set of enzymes used, no differences were detected between strains of subgenus B isolated from the conjunctiva or the respiratory tract.

While genome type Ad7b has been identified among isolates collected in the South Cone of South America [Kajon and Vicente Suarez, 1990; Kajon and Wadell, 1992; Kajon et al., 1994; 1996], Ad7e seems so far to be unique to this part of the continent, having been isolated also in Australia [Li and Wadell, 1986].

These results and those previously reported by Baroni de Moraes et al. [1997] for Ad7 strains collected in Rio de Janeiro between 1980 and 1991, suggest that Ad7b, the currently predominant virulent genome type of serotype 7 in Europe and North America, and Ad7e have shown an alternating appearance over the years in this region. A larger number of strains and a continuous sampling are required in order to detect substitutions for one DNA variant by another and also to more accurately determine whether Rio de Janeiro and Belem share the same molecular epidemiological characteristics for subgenus B adenovirus infections. All the Brazilian isolates of serotype 3 analyzed were found to belong to genotype 3p1. This genomic variant differing from the prototype in its Hpa I profile, has been previously identified by Li and Wadell [1988] among Ad strains isolated in Holland, South Africa, and Brazil. The prototypelike DNA variants of serotype 3 isolated in the South Cone of South America belong to genome types 3p2 [Kajon et al., 1994; 1996] and 3p3 [Kajon and Wadell, 1992].

In spite of the relatively small size of the sample analyzed, the results of this study confirm the impor-

TABLE II. Characteristics of Identified Genome Types of Subgenus C

Serotype	Genome type	Enzyme code[a] BamH I	Bgl II	BstE II	EcoRI	Hind III	Kpn I	Sma I	No. of isolates	Years of circulation	Place of isolation
1 (n = 14)	D1	1	1	1	1	1	1	1	1	92	Belem
	D7	2	1	2	1	1	1	1	2	86/87	Belem/Rio
	D10	2	1	3	1	1	1	1	10	83/84/85/87/90/91	Belem/Rio
	New **a**	1	1	1	1	new I	1	1	1	90	Rio
2 (n = 29)	D2	1	1	2	1	1	1	1	1	86	Rio
	D5	1	1	2	2	3	1	3	4	89/91	Rio
	D25	1	1	2	2	3	1	6	5	83/85/87/90	Belem/Rio
	New **a**	3	1	2	1	1	1	1	1	82	Rio
	New **b**	new II	1	3	2	3	new IV	3	1	82	Rio
	New **c**	3	1	3	1	1	new IV	3	2	83	Belem
	New **d**	3	1	2	1	1	new IV	3	1	83	Belem
	New **e**	1	1	1	2	2	3	6	1	85	Rio
	New **f**	1	1	2	1	1	1	1	1	85	Rio
	New **g**	3	1	1	1	1	1	1	1	86	Rio
	New **h**	1	1	new III	1	5	new V	1	1	87	Belem
	New **i**	1	1	new III	2	3	8	3	1	87	Rio
	New **j**	1	1	1	2	3	1	6	3	87/90/95	Belem/Rio
	New **k**	1	1	1	2	2	1	6	2	90	Rio
	New **l**	1	1	1	3	2	1	6	3	90/91	Rio
	New **m**	1	7	2	2	3	1	6	1	91	Rio
5 (n = 16)	D38	2	2	2	2	2	2	1	4	82/87/94	Belem/Rio
	New[b]	2	2	4	2	2	2	1	8	83/85/90/94/95	Belem/Rio
	New **a**	1	2	2	2	2	2	1	1	87	Belem
	New **b**	2	7	7	1	1	4	1	1	90	Rio
	New **c**	2	2	4	1	1	2	1	1	90	Rio
	New **d**	2	2	4	2	new VI	2	1	1	94	Belem

[a]**new I-VI:** restriction patterns not previously described.
[b]Recently identified among Ad5 strains isolated in the south cone of South America (Kajon et al., 1996).

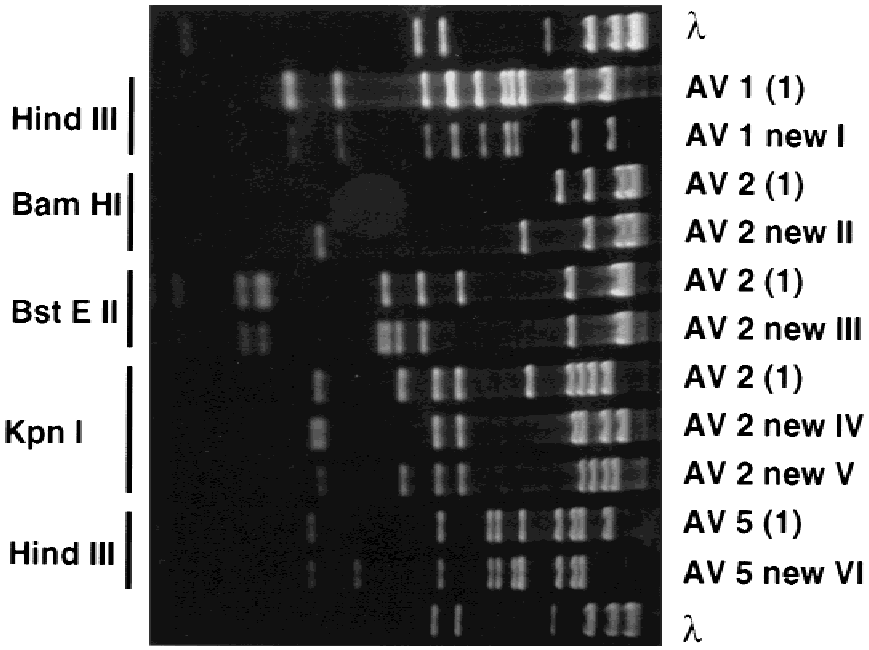

Fig. 2. Novel restriction profiles (new I-VI) obtained with Bam HI, BstE II, Hind III and Kpn I for Ad1, Ad2, and Ad5 strains. AV1 (1), AV2 (1), and AV5 (1) are the profiles for the corresponding prototype strains. Lambda DNA digested with Hind III was used as a molecular weight marker.

tant genetic variability previously observed by other authors for members of subgenus C in a given region [Adrian et al., 1989, 1990]. No conclusions can be drawn about the patterns of circulation or seasonality since only a few isolates of each genome type were detected and they were widely scattered over the time period covered by the study.

To the best of our knowledge, the isolation of strains

corresponding to genome types **new a** of Ad1, **new a–m** of Ad2 and **new a–d** of Ad5 has not been reported for other areas of the world, including the neighboring Argentina and Uruguay. The DNA variant of Ad5 denominated "New *" in this article was also identified among strains circulating in Argentina between 1991 and 1993 [Kajon et al., 1996]. It would be interesting to determine whether the south of Brazil has a different molecular epidemiology of adenovirus infections and whether it resembles more closely that described for Argentina, Chile, and Uruguay.

Several reports have shown data strongly suggesting the existence of geographic variation among human adenoviruses, especially Ad3 and Ad7. The continuous surveillance of adenovirus infections and the characterization of viral isolates is essential to determine which the most prevalent virulent serotypes and genomic variants are in a given region since the observed genetic variability might have an impact on the design of effective preventive programs.

ACKNOWLEDGMENTS

We thank Terezinha R. Pereira for technical assistance, Hermann Schatzmayr for his continuous support and encouragement, and Dr. Katherine Spindler for allowing this work to be completed in her laboratory. A.E.K. received a visiting researcher fellowship from the Oswaldo Cruz Institute.

REFERENCES

Adrian T, Best B, Wigand R. 1985. A proposal for naming adenovirus genome types exemplified by adenovirus type 6. J Gen Virol 66: 2685–2691.

Adrian T, Wigand R, Knocke KW, Schäfer G, Grundmann M. 1989. Genome type analysis of adenoviruses: isolates from one year from the Hannover area. Arch Virol 105:89–101.

Adrian T, Sassinek J, Wigand R. 1990. Genome type analysis of 480 isolates of adenovirus types 1, 2 and 5. Arch Virol 112:235–248.

Baroni de Moraes MT, Leite JPG, Siqueira MM, Portes SR, Krawczuk MM, Nascimento JP. 1997. Genomic characterization of adenovirus serotype 7 isolated in Brazil from acute respiratory disease patients during the period from 1980 to 1991. Rev Inst Med Trop Sao Paulo 39:185–189.

Garcia AGP, Fonseca MEF, Debonis M, Ramos HIB, Ferro ZPA, Nascimento JP. 1993. Morphological and virological studies in 6 autopsies of children with adenovirus pneumonia. Mem Inst Oswaldo Cruz 88:141–147.

Gomes SA, Candeias JAN, Monteiro SP, Pereira HG, Niel C. 1989. New genome types of adenovirus types 1, 3 and 5 isolated from stools of children in Brazil. J Clin Microbiol 27:1022–1026.

Harsi CM, Rolim DP, Gomes SA, Gilio AE, Stewien KE, Baldacci ER, Candeias JAN. 1995. Adenovirus genome types isolated from stools of children with gastroenteritis in Sao Paulo, Brazil. J Med Virol 45:127–134.

Kajon A, Vicente Suarez M. 1990. Molecular epidemiology of adenoviruses isolated from hospitalized children with severe lower acute respiratory infection in Santiago, Chile. J Med Virol 30:294–297.

Kajon A, Wadell G. 1992. Molecular epidemiology of adenoviruses associated with acute lower respiratory disease in children in Buenos Aires, Argentina (1984–1988). J Med Virol 36:292–297.

Kajon A, Larrañaga C, Suarez M, Wadell G, Avendaño LF. 1994. Genome type analysis of Chilean adenovirus strains isolated in a children's hospital between 1988 and 1990. J Med Virol 42:16–21.

Kajon AE, Mistchenko AS, Videla C, Hortal M, Wadell G, Avendaño LF. 1996. Molecular epidemiology of acute lower respiratory infections of children in the South Cone of South America (1991–1994). J Med Virol 48:151–156.

Li Q-G, Wadell G. 1986. Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. J Virol 60:331–335.

Li Q-G, Wadell G. 1988. Comparison of 17 different genome types of adenovirus 3 identified among strains recovered from six continents. J Clin Microbiol 26:1009–1015.

Murtagh P, Kajon A. "Chronic pulmonary sequelae of adenovirus infection" Pediatric Pulmonology, 1997, Supplement 16:150–151.

Murtagh P, Cerqueiro C, Halac A, Avila M, Kajon A. 1993. Adenovirus type 7h respiratory infections: a report of 29 cases of acute lower respiratory disease. Acta Paediatr 82:557–561.

Nascimento JP, Siqueira MM, Sutmoller F, Krawczuk MM, Farias V, Ferreira V, Rodriguez MJ. 1991. Longitudinal study of acute respiratory diseases in Rio de Janeiro: occurrence of respiratory viruses during four consecutive years. Rev Inst Med Trop Sao Paulo. 33:287–296.

Niel C, Moraes MTB, Mistchenko AS, Leite JPG, Gomes SA. 1991. Restriction site mapping of four genome types of adenovirus 3 and 7 isolated in South America. J Med Virol 33:123–127.

Rowe WP, Huebner RJ, Gilmore LK, Parrot LH, Ward TG. 1953. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc Soc Exp Biol Med 97:465–470.

Schmitz H, Wigand R, Heinrich W. 1983. Worldwide epidemiology of human adenovirus infections. Am J Epidemiol 117:455–466.

Schnurr DP, Dondero ME. 1993. Two new candidate adenovirus serotypes. Intervirology 36:79–83.

Shenk T. 1995. Adenoviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, editors. Fields' Virology, Vol 2. New York: Lippincott-Raven, p 2111–2148.

Shinagawa M, Matsuda A, Ishiyama T, Goto H, Sato G. 1983. A rapid and simple method for preparation of adenovirus DNA from infected cells. Microbiol Immunol 27:817–822.

Wu E, Martinez V, Alvarez AM, Larrañaga C, Vela H. 1990. Casos fatales de infeccion por adenovirus. Rev Chiena Pediatr 61:177–184.